IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No. : **10/621,760**

Applicants : **David L. Lewis et al.**

Filed : **07/17/2003**

Art Unit : **1633**

Examiner : **Popa, Ileana**

Docket No. : **Mirus.030.09.2**

For: **Compositions and Processes Using siRNA, Amphipathic Compounds and Polycations**

Commissioner of Patents
PO Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. ' 1.132

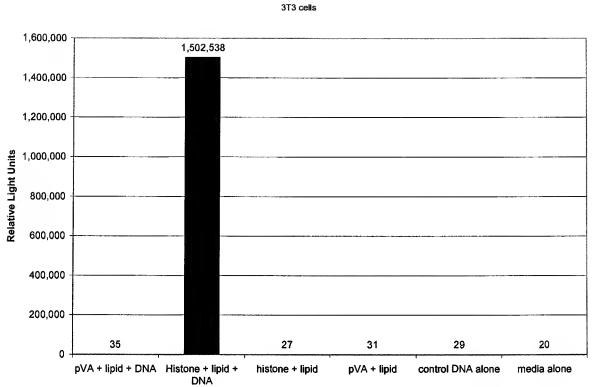
Dear Commissioner

I, James E. Hagstrom, hereby declare as follows:

1. I am an inventor of the above captioned application and on the reference patent U.S. 5,744,335.

2. I submit, with this Response, experimental material illustrating the following:
   a) histone + MC798 (amphipathic compound) forms an effective plasmid DNA delivery agent, while polyvinylamine + MC798 does *not* form an effective plasmid DNA delivery agent.

   The lipid used in the following examples was MC798 (structure represented in FIG. 3C of the above captioned application). Histone, but not polyvinylamine (pVA), when combined with an amphipathic compound such as MC798, forms an effective plasmid DNA *in vitro* delivery agent. Complexes containing plasmid DNA, histone or pVA, and amphipathic compound were prepared as described in U.S. 5,744,335.


3T3 cells
Relative Light Units
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1,400,000
1,200,000
1,000,000
800,000
600,000
400,000
200,000
0
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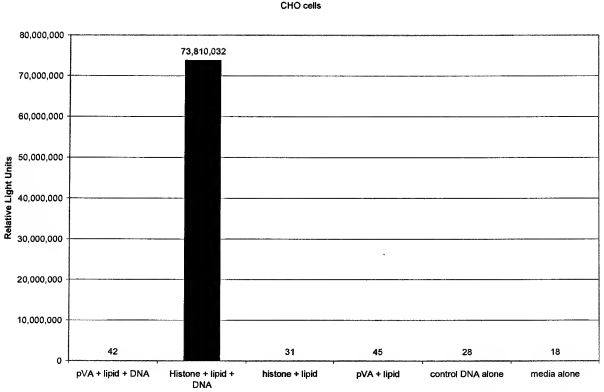
35
27
31
29
20
pVA + lipid + DNA
Histone + lipid + DNA
histone + lipid
pVA + lipid
control DNA alone
media alone


CHO cells
Relative Light Units
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70,000,000
60,000,000
50,000,000
40,000,000
30,000,000
20,000,000
10,000,000
0
73,810,032
42
31
45
28
18
pVA + lipid + DNA
Histone + lipid + DNA
histone + lipid
pVA + lipid
control DNA alone
media alone

b) polyvinylamine + MC798 forms an effective siRNA delivery agent, while histone + MC798 does *not* form an effective siRNA delivery agent.

Cos7 cells, 293 cells, or 3T3 cells were initially transfected with a plasmid DNAs encoding the Firefly luciferase gene and the Renilla luciferase genes, resulting in cells that expressed these two luciferase proteins. These cells were then transfected with 5 nM Firefly luciferase siRNA using either ethoxylated polyethyleneimine (ePEI) + MC798 or histone + MC798. siRNA-containing complexes were prepared as taught in the above captioned application.

Successful delivery of the Firefly luciferase siRNA to cells expressing the Firefly luciferase gene results in knockdown (inhibition) of Firefly luciferase gene expression. Thus, delivery of Firefly luciferase siRNA directly correlates with knockdown of Firefly luciferase gene expression. Higher knockdown means more efficient delivery, while lower knockdown means less efficient or no delivery. As shown in the table below, siRNA was efficiently delivered with ePEI + MC798: 71% to 90% knockdown. In contrast, histone + MC798 was not an effective siRNA delivery reagent: 0% knockdown.

transfection reagent	Luciferase expression	% knockdown
none	1.0000	0.0000
ePEI + MC798	0.0960	0.9040
histone +MC798 (ratio 1)	1.0760	−0.0760
histone + MC798 (ratio 2)	1.1820	−0.1820
histone + MC198 (ratio 3)	1.1980	−0.1980
ePEI + MC798	0.2830	0.7170
histone + MC798	1.0398	−0.0398
ePEI + MC798	0.2222	0.7778
histone + MC798	1.0344	−0.0344

In this experiment, ePEI + MC798 was used as a control, to demonstrate that the siRNA is effective in inhibiting gene expression. As shown in the above captioned application, both ePEI (Example 2A, B, C, E, F) and polyvinylamine (Example 3A, see especially

tables 6 and 7), when combined with amphipathic compound, such as MC798, are effective siRNA delivery agents. However, the combination of histone + MC798 as taught by U.S. Patent 5,744,335, does not effectively delivery siRNA to cells *in vitro* (no inhibition of luciferase expression in above experiment).

3. Compound #4 of U.S. 5,744,335 is the reduced form of compound MC798 (represented in FIG. 3C of the above captioned application). Both compounds are 1,4 disubstituted piperazines. It is known by me that the composition of Histone H1 + MC798 is a more effective plasmid DNA delivery agent than the composition of Histone H1 + compound #4. Substitution of compound #4 in the above experiments would be expected to produce similar but less efficient transfection results.

4. The above data are reproduced from Declarations signed by me and filed on 12/11/2006, 07/02/2007 and 12/05/2007. In the previously filed Declarations, I incorrectly stated, without deceptive intent, that the amphipathic compound used in the above experiments was identical to that used by U.S. Patent 5,744,335. The amphipathic compound used in the above experimental data is correctly identified as compound MC798.

5. Size data for histone/MC798/DNA particles complexes were determined by 90° light scattering at 532 nm using a Brookhaven Instruments Corporation, ZetaPlus Particle Sizer, I90 (software ver. 4.02). Complexes were prepared under typical transfection conditions: isotonic glucose and 150 mM salt at 25°C. Histone H1/MC798/plasmid DNA complexes were found to have an effect diameter of ~963.9 nm. We have found that larger particles are frequently more effective *in vitro* DNA transfection complexes than smaller complexes. Thus, small complex size is not predictive of efficient *in vitro* DNA transfection capability.

6. The material is consistent with the specification as filed and only methods described in the specification have been used. No new matter was used in the experiments.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

5/5/08

Dr. James E. Hagstrom Date